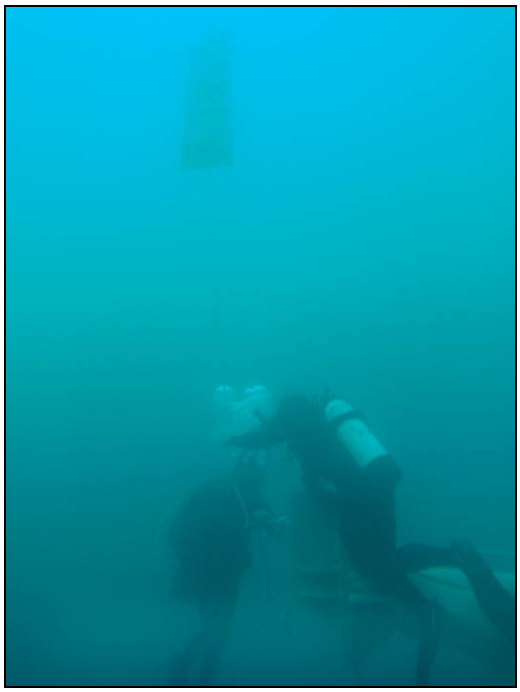
WorleyParsons
resources & energy

EcoNomics

NSW CROWN LANDS (A DIVISION OF THE NSW DEPARTMENT OF PRIMARY INDUSTRIES)

Ex-HMAS ADELAIDE Artificial Reef

Bioaccumulation Study



301020-03410 - DRAFT REV C

28 June 2011

Infrastructure & Environment
8-14 Telford Street
Newcastle East NSW 2300 Australia
Tel: +61 2 4907 5300
Fax: +61 2 4907 5333
www.worleyparsons.com
WorleyParsons Services Pty Ltd
ABN 61 001 279 812

Executive Summary

The Ex-HMAS ADELAIDE was scuttled off the coast of Terrigal and Avoca Beach on 13 April 2011 for the purpose of creating an artificial reef and recreational dive site. In accordance with the Artificial Reef Permit, NSW Crown Lands (a division of the Department of Primary industries) must implement the approved *Long Term Management and Monitoring Plan* (LTMMP) which includes a Bioaccumulation Study. The objective of the *Bioaccumulation Study* is to determine whether it is likely that resident marine organisms on the Ex-HMAS ADELAIDE (i.e. organisms in direct contact with the vessel) are likely to be affected by degradation of the zinc chromate paint that may have originally been applied on the vessel.

For the *Bioaccumulation Study* the Blue Mussel, *Mytilus edulis*, was used as the test organism. Mussels were deployed to three impact sites on the Ex-HMAS ADELAIDE and two reference sites on mooring lines approximately 35 m from the vessel. Mussel samples collected directly from the source (i.e. the aquaculture facility) were also tested to determine baseline levels of contaminants. In the month following scuttling most of the dive moorings and special marker buoys around the Ex-HMAS ADELAIDE were lost or displaced due to extreme weather conditions and suspected tampering. As a result, mussel bags from the reference sites were never recovered.

Mussels were retrieved from the impact sites after a six weeks deployment period. Mean values were determined for concentrations of chromium, zinc and lead in mussels from each impact site. These values were compared to pre deployment concentrations using analysis of variance (ANOVA). Unfortunately, the comparison of primary interest between the three impact sites and two reference sites was not possible.

Overall, there were significant differences in metals concentrations in mussel tissue between the zero controls and impact sites. *Post hoc* testing identified significant differences in the concentrations of chromium and lead, but no significant differences in zinc concentrations. Although an increase in metal concentrations has been observed between the transplanted mussels and the mussels placed near the vessel, the significant increase noted for lead and chromium cannot be directly attributed to the presence of the vessel without consideration of reference concentrations. Without data from the two reference areas, results are confounded.

Disclaimer

PROJECT 301020-03410 - EX-HMAS ADELAIDE ARTIFICIAL REEF

DESCRIPTION	ORIG	REVIEW	WORLEY-PARSONS APPROVAL	DATE	CLIENT APPROVAL	DATE
DRAFT Issued for internal review	Dr K Newton	H Houridis		24 June 2011	N/A	
DRAFT Issued for internal review	Dr K Newton	H Houridis	H Houridis	27 June 2011	N/A	27 June 2011
DRAFT Issued for client review	Dr K Newton			28 June 2011		

CONTENTS

Tables

Figures

Appendices

NSW CROWN LANDS (A DIVISION OF THE NSW DEPARTMENT OF PRIMARY INDUSTRIES)
EX-HMAS ADELAIDE ARTIFICIAL REEF
BIOACCUMULATION STUDY

1. INTRODUCTION

The Ex-HMAS ADELAIDE was a long-range escort frigate which was scuttled off the coast of Terrigal and Avoca Beach on Wednesday 13 April 2011 for the purpose of creating an artificial reef and recreational dive site. An Artificial Reef Permit (SD2008/1062) was issued by the Department of Environment, Water, Heritage and the Arts (DEWHA), under the *Environment Protection (Sea Dumping) Act* 1981, in March 2010. In accordance with the Artificial Reef Permit, NSW Crown Lands (a division of the Department of Primary industries) must implement the approved *Long Term Management and Monitoring Plan (LTMMP)*.

The purpose of the LTMMP is to provide for the post-scuttling management and monitoring of the Ex-HMAS ADELAIDE Artificial Reef and covers monitoring for the first five years post-scuttling. The LTMMP contains provision for review, based on the results of the monitoring.

The LTMMP includes requirements to undertake and report changes in the environmental conditions on and around the artificial reef. Specifically, the environmental monitoring includes the following:

- Reef communities survey;
- Sediment movement;
- Sediment quality; and
- Bioaccumulation study.

The results of the initial *Bioaccumulation Study* are provided in this report.

1.1 Background

Zinc chromate was routinely used as an anticorrosive application on the topside of naval vessels. It is understood that the more recent coating formulations on the Ex-HMAS ADELAIDE did not contain chromium salts. After scuttling the zinc chromate paint is expected be subjected to corrosion and microbial attack and will likely deteriorate over time. While the environmental fate of zinc chromate in the marine environment is not well understood, it is assumed that the metal constituents zinc and chromium will be liberated into the marine environment through a processes involving dissolution and flaking. The zinc and chromium will potentially affect marine organisms that foul or live directly on the vessel through a process of bioaccumulation into their tissues.

Biomonitoring of marine fouling organisms on the Ex-HMAS ADELAIDE was proposed in the LTMMP to investigate the potential for bioaccumulation of the products of zinc chromate degradation in the

tissues of resident biota. However, it is acknowledged that the development of fouling communities on the vessel may take some time, so an active biomonitoring study has been proposed until there is sufficient growth / resident biota for direct testing to occur. Active biomonitoring involves the collection of marine organisms from a non impacted / comparatively clean location (e.g. an aquaculture facility) and translocation to a test site / potentially impacted site (e.g. the Ex-HMAS ADELAIDE) for a fixed period of deployment. Following deployment for this fixed period, laboratory analysis is undertaken to determine the concentration of any contaminants of interest that may have accumulated in the tissues of the selected organism.

For the *Bioaccumulation Study* on the Ex-HMAS ADELAIDE the LTMMP proposed that the Blue Mussel, *Mytilus edulis*, be used as the test organism. The contaminant of concern was zinc chromate (N.B. zinc chromate is tested for in the laboratory as chromium and zinc, the products of zinc chromate degradation). In addition, although not stipulated in the LTMMP, lead was also tested for in mussel tissues due to community concern over the previous use of lead paint on the vessel.

The LTMMP required that mussels for the initial *Bioaccumulation Study* be deployed within a month post scuttling. Additional sampling and analysis will be undertaken 12 months post scuttling, with active biomonitoring planned to continue if concentrations of contaminants are elevated or of concern, and if insufficient fouling biota have colonised the vessel to allow for *in situ* sampling. Two control sites (located on the moorings lines located around the vessel) and three impact sites (on the vessel) are required, with mussels being deployed for a period of 6 – 8 weeks.

1.2 Location of the Dive Site

The Ex-HMAS ADELAIDE is located in Bulbararing Bay, between Avoca Beach and Terrigal Headland, on the NSW Central Coast. The ship is located approximately 1.4 km from Terrigal Headland and 1.9 km from Avoca Beach (**Figure 1.1**) with a depth of water over the main mast of 8.02 m LAT (personal communication NSW Crown Lands 2011). **Table 1.1** provides the scuttling co-ordinates for the vessel.

Table 1.1 Co-ordinates of the scuttling location for the Ex-HMAS ADELAIDE.

Latitude / Longitude	Northing / Easting (MGA 94)
Latitude (south): 33˚27.91’	Northing (MGA 94): 6,296,076.969
Longitude (east): 151˚27.38’	Easting (MGA 94): 356,551.686

After site selection studies were completed it was determined that the vessel would be scuttled with an ESE orientation (112º), so that the bow would be facing into the general direction of the largest waves (coming from the SE, ESE and S). As sunk, the vessel is oriented at 116º and is generally upright, with a small list of 2.5 degrees to port (personal communication, Land and Property

Management Authority 2011). The *Review of Environmental Factors* (REF) undertaken for the dive site describes the final scuttling site as being in 32 m of water at Lowest Astronomical Tide (LAT) (WorleyParsons 2009a). After scuttling, the depth of water over the main mast is 8.02 m LAT (personal communication LPMA 2011).

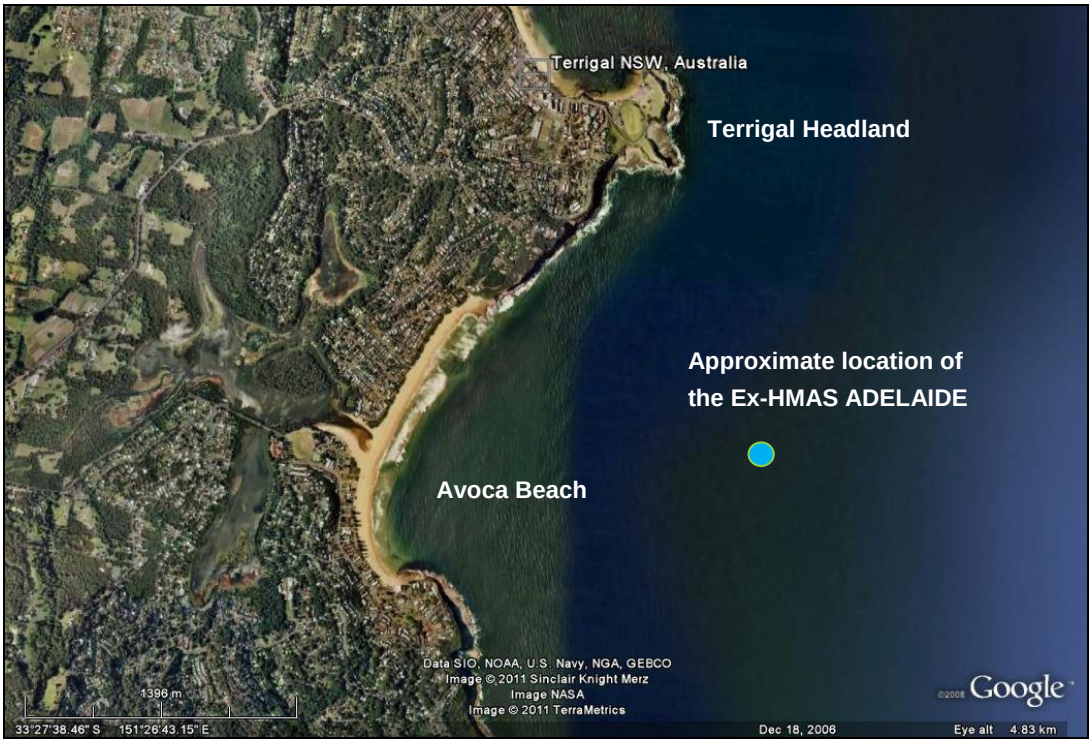


Figure 1.1 Approximate location of the Ex-HMAS ADELAIDE artificial dive reef.

1.3 Study Objective

The objective of the *Bioaccumulation Study* is to determine whether it is likely that resident marine organisms on the Ex-HMAS ADELAIDE (i.e. organisms in direct contact with the vessel) are likely to be affected by degradation of the zinc chromate paint that may have originally been applied on the aluminium alloy vessel.

Section 2 of this report outlines the study methods and **Section 3** provides the results of the *Sediment Quality Survey*. In **Section 4** a discussion of the survey results is provided.

2. METHODS

For the initial *Bioaccumulation Study,* the LTMMP required that mussels be deployed within a month post scuttling. Deployment to reference and impact sites was undertaken on Wednesday19th April 2011; one week after the Ex-HMAS ADELAIDE was sunk. The technical details regarding the mussel bioaccumulation study are based de Kock and Kramer (1994) and the survey design on a previous biomonitoring program undertaken by Haynes and Toohey (1998).

2.1 Mussels

Blue Mussels were sourced from an aquaculture facility, Eden Sea Farms, Eden, located in southern NSW. This is the only aquaculture facility in NSW from which live Blue Mussels are available. All mussels were obtained immediately prior to deployment and were in the size class of 50 to 60 mm. Mussels were transported overnight from the aquaculture facility to the study site in a dark cool Styrofoam esky which contained a small quantity of ice to ensure that mussels survived but were not frozen during transport. Any mussels that were dead on arrival were discarded and not used in the study.

2.2 Experimental Setup

2.2.1 Mussel Bags

Mussels were placed in specially constructed mussel bags, constructed using black UV stable oyster mesh, with a mesh size of 20 x 20 mm to allow sufficient water flow but prevent loss of samples (see **Figure 2.1**). The mussel bags were attached to the vessel (impact sites) and mooring lines (reference sites) using a combination of 15 mm marine grade rope and black cable ties. The mussel bags attached to the vessel were also equipped with a small buoy to ensure the bags were suspended approximately 2 m above the structure into the water column, allowing flow of water for the mussels to survive (see **Figure 2.2**).

2.2.2 Replication

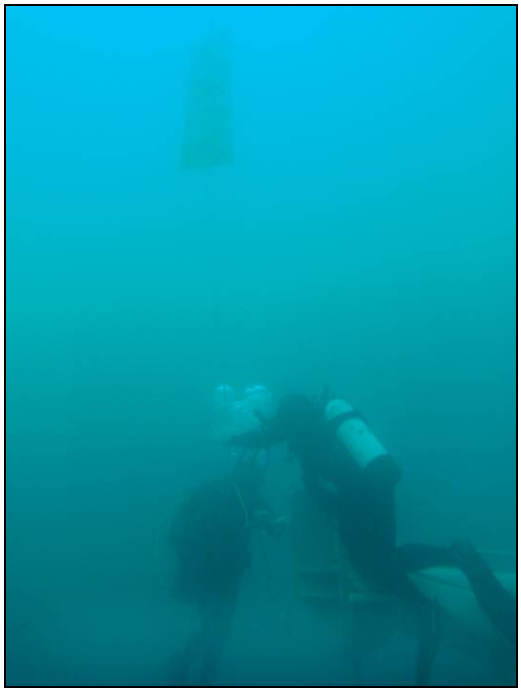
Three mussel bags were deployed per site. Within each mussel bag, at least 30 mussels were deployed to insure against losses and ensure that sufficient numbers of mussels were available for laboratory analysis (i.e. 15 to 20 mussels per cage per site were required).

2.2.3 Diving

All mussel bags were deployed and retrieved by commercially qualified SCUBA divers (**Figure 2.2**).

Figure 2.1 Mussel bags were constructed with UV stable oyster mesh.

a.



b.

Figure 2.2 a) example of the mussel bag setup on the Ex-HMAS ADELAIDE and b) divers installing the mussel bags.

2.3 Mussel Deployment Sites

Mussels were deployed to three impact sites (on the Ex-HMAS ADELAIDE) and two reference sites (on mooring lines around the vessel). Mussel samples collected directly from the source (i.e. the aquaculture facility) were also tested to determine baseline levels of contaminants.

2.3.1 Impact Sites

Three impact sites were selected at the bow, mid section and stern of the Ex-HMAS ADELAIDE at a depth of approximately ~ 20 m (**Figure 2.3**). Three mussel bags were deployed at each of these sites, with bags held at a height of approximately 2 m off the main deck using a small Styrofoam subsurface buoy (see **Figure 2.2**). All mussels were deployed to attachment points on the port side of the vessel.

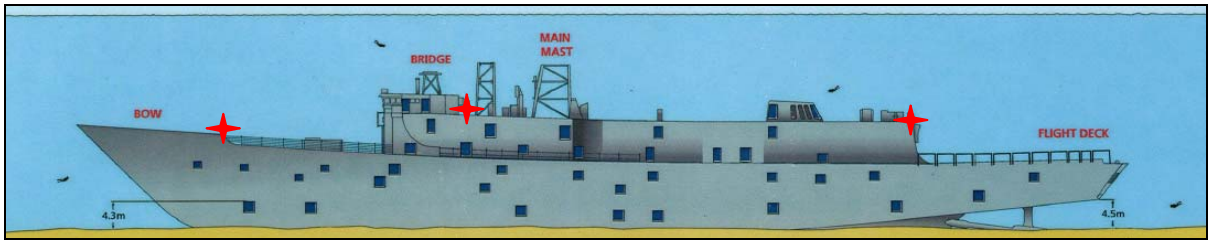


Figure 2.3 Approximate location of the attachment points (✛) for mussel bags on the vessel.

2.3.2 Reference / Control Sites

Two reference sites were located on mooring lines near the Ex-HMAS ADELAIDE. During mussel deployment, the six dive moorings were still being installed by McMahon Services so mussel bags were attached to the mooring lines of two special marker buoys for which installation had been completed (see **Figure 2.4**). The two special marker buoys are located at a distance of approximately 35 m from the bow and stern of the vessel (personal communication, Crown Lands, 2011). Attachment of mussel bags to the special marker buoy mooring lines was also considered to be preferable over attachment to the dive mooring lines as they were much stronger and there would be considerably less potential for damage or tampering by divers ascending or descending the lines. Depth of attachment at the reference sites was the same as for the impact sites (i.e. ~ 20 m).

However, in the month following installation, the dive moorings and special marker buoys around the Ex-HMAS ADELAIDE, were lost or displaced due to extreme weather conditions and suspected tampering (**Figure 2.4**). Special marker buoys were found without the mooring lines or mussel bags attached. As a result, mussel bags from the reference sites were lost. Since deployment of further mussel bags at a different time period would not have produced comparable results, analysis for the initial *Bioaccumulation Study* could only be undertaken between the impact site and zero control mussels.


a.


b.

Figure 2.4 a) the special marker buoy mooring lines were used as reference sites; b) special marker buoys and a dive mooring tied together after they were lost from the site.

2.3.3 Zero Controls

Tissue samples of mussels from the original source need to be tested. These samples will act as a "zero control" and determine the background levels of zinc, chromium and lead in the mussel tissue prior to deployment to the site. Zero control samples were taken directly from the batch of mussels supplied by the Eden Sea Farms aquaculture facility. Five replicate zero control samples consisting of 15 mussels each was sent for analysis to determine the baseline concentrations of chromium, zinc and lead.

2.4 Analysis of Tissues

2.4.1 Sub Sampling in Field

Mussels were retrieved from the vessel using SCUBA, six weeks post deployment, in accordance with the LTMMP. On land, the mussel bags were opened and any mussels which had not survived were discarded. A total of 15 mussels were selected from each of the mussel bags and placed into snap-lock freezer bags labeled as follows:

- Bow 1, 2, 3
- Mid 1, 2, 3
- Stern 1, 2, 3

Mussel samples were kept on ice in the dark for transportation to the analytical laboratory.

2.4.2 Laboratory Sampling

Under the LTMMP, sampling and analysis of mussel tissue is required to be undertaken by a NATA accredited laboratory and results are required to be reported in dry weight (N.B. wet weight reporting is needed if comparison with food standards is required however this was not the case).

Samples were analysed by the NATA accredited laboratory Advanced Analytical. In the laboratory, tissue from each of the 15 mussels making up each sample were freeze dried and homogenised into one composite sample to reduce the effect of intraspecific variability between individuals. These samples were analysed for the metals chromium and zinc (the constituents of zinc chromate) and for lead. Results were presented as dry weight in mg/kg. Laboratory QA / QC was undertaken. Laboratory Sample Receipts and original Reports of Analysis from the zero control and impact site mussels are provided in **Appendix 1**.

2.5 Data Analysis

Mean values were determined for concentrations of chromium, zinc and lead for each impact site individually, for the zero control sites combined and impact sites combined. These values were compared. Analysis of Variance (ANOVA) was also used to determine any spatial differences between the concentrations of chromium, zinc and lead in mussel tissues between the three impact sites and also differences between concentrations in the zero control and impact site mussel tissues. Prior to this the data were tested for normality. Data were normally distributed and variances were homogenous so no data transformation was necessary (see **Appendix 2**). The comparison of primary interest between the three impact sites and two reference sites was not possible. *Statistica* Version 5 was used to undertake all data analysis.

3. RESULTS

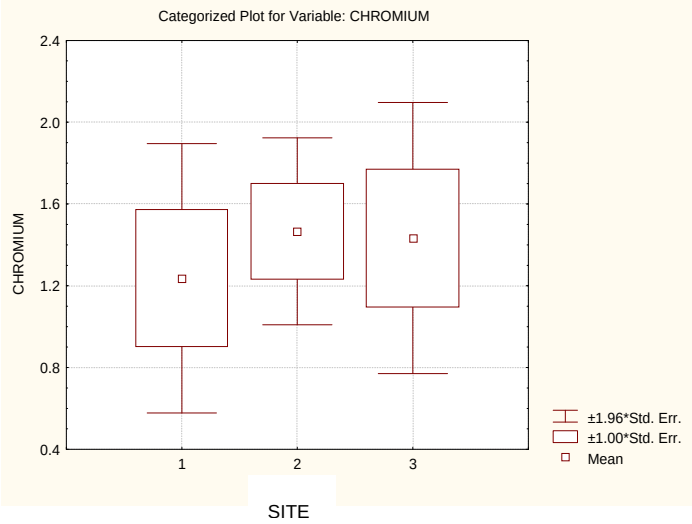
Concentrations of chromium, zinc and lead in the tissue of mussels from the zero control and impact sites were determined in the laboratory. Original laboratory reports are provided in **Appendix 1**. Results of analysis are provided below. **Appendix 2** provides outputs of the statistical analysis.

3.1 Zero Controls (Background Tissue Concentrations)

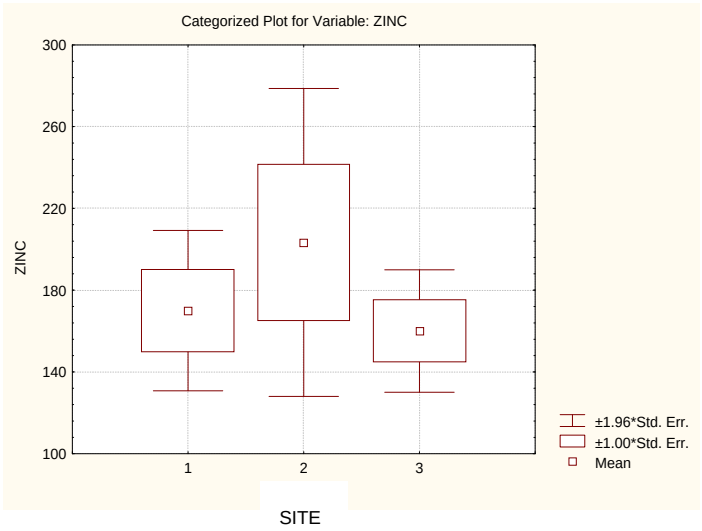
Concentrations of chromium, lead and zinc were detected above the LOR in all zero control samples and little variation was found between samples. The mean chromium concentration in mussel tissues from zero controls was 0.67 mg/kg with a standard deviation of 0.1 mg/kg. Mean zinc concentration in the in the zero controls was 152 mg/kg with a standard deviation of 29.5 mg/kg. Lead had a mean concentration in zero controls of 0.23 mg/kg with a standard deviation of 0.04 mg/kg.

3.2 Impact Sites

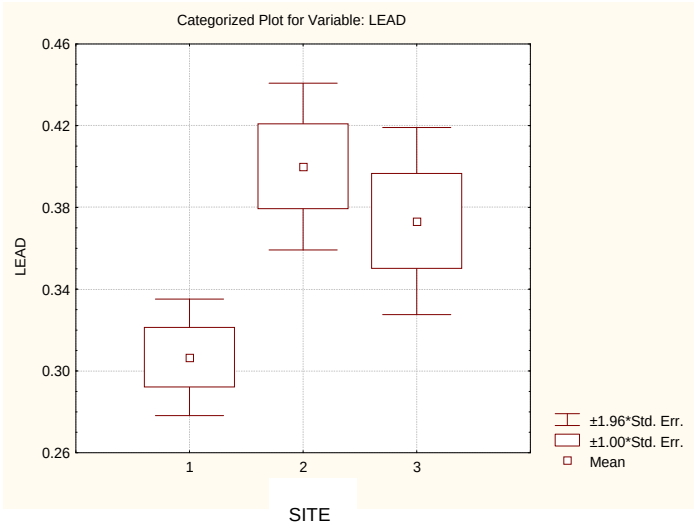
Concentrations of chromium, lead and zinc were detected above the level of reporting (LOR) in all samples analysed from the impact sites. A comparison of mean concentrations of metals in tissue between the three impact sites found that concentrations from the bow, stern and mid section were generally similar (**Figure 3.1**, **Table 3.1**). When data was combined, the impact samples had a mean chromium concentration of 1.4 mg/kg with a standard deviation of 0.47 mg/kg. Zinc was found to have a mean value of 178 mg/kg (standard deviation of 44.4 mg/kg). The mean concentration of lead in mussel tissues from the impact sites was 0.36 mg/kg and standard error was 0.05 mg/kg (**Table 3.2**). Overall, there were no significant differences in metal concentrations in tissues between the three impact sites at 6 weeks ($F_{6, 8}$ = 1.67, p = 0.244) (**Figure 3.1**, **Appendix 2**).



a. Chromium



b. Zinc



c. Lead

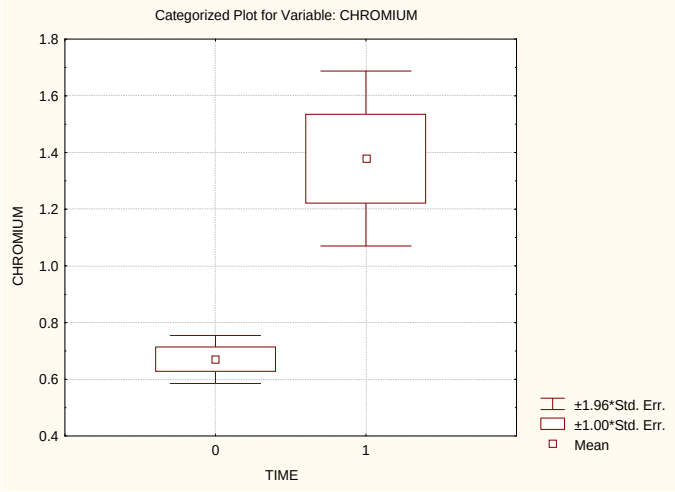
Figure 3.1 Box plots showing differences in mean values for chromium, zinc and lead concentrations at the three impact locations (1 = bow, 2 = mid, 3 = stern).

Table 3.1 Metal concentrations in mussel tissue at the impact sites.

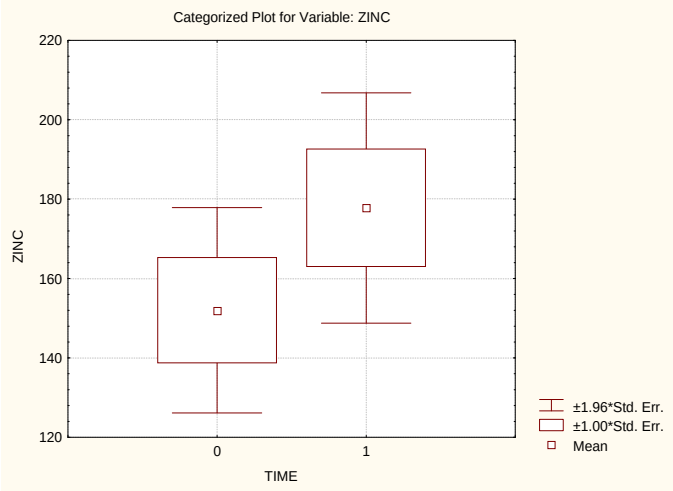
Sample ID / Date Sampled			Chromium	Lead	Zinc
Unit (dry weight)			mg/kg	mg/kg	mg/kg
Level Of Reporting (LOR)			0.1	0.1	0.2
IMPACT SITES	Bow1	30/05/2011	1.9	0.33	150
	Bow2	30/05/2011	0.81	0.31	150
	Bow3	30/05/2011	1	0.28	210
	Mean		1.2	0.31	170
	StDev		0.58	0.03	35
	Median		1.0	0.31	150
	Min		0.81	0.28	150
	Max		1.9	0.33	210
	Mid1	30/05/2011	1.4	0.36	130
	Mid2	30/05/2011	1.1	0.43	260
	Mid3	30/05/2011	1.9	0.41	220
	Mean		1.5	0.4	203
	StDev		0.40	0.04	66.6
	Median		1.4	0.41	220
	Min		1.1	0.36	130
	Max		1.9	0.43	260
	Stern1	30/05/2011	2.1	0.41	170
	Stern2	30/05/2011	1.2	0.38	180
	Stern3	30/05/2011	1	0.33	130
	Mean		1.4	0.37	160
	StDev		0.59	0.04	26.5
	Median		1.2	0.38	170
	Min		1.0	0.33	130
	Max		2.1	0.41	180

3.3 Comparison between Impact Site and Zero Controls

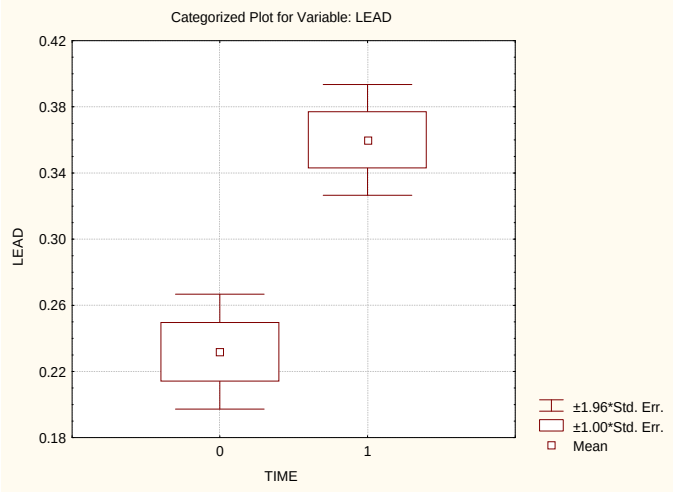
The mean concentration of all metals was higher at the impact sites that the zero control samples (**Figure 3.2**, **Table 3.2**). One way ANOVA was undertaken to assess the differences between concentrations of chromium, zinc and lead between the zero control and impact sites. Overall, there were significant differences in metals concentrations in mussel tissue between the zero controls and impact sites ($F_{3, 10} = 7.22$, $p = 0.007$). *Post hoc* testing showed that these differences were caused by differences in the concentrations of chromium and lead, however, no differences were apparent for zinc concentrations (refer to **Appendix 2**).



a. Chromium



b. Zinc



c. Lead

Figure 3.2 Box plots showing differences in mean values for chromium, zinc and lead concentrations between the zero control and impact samples (0 = zero control, 1 = impact).

Table 3.2 Metal concentrations in mussel tissue for zero controls and impact sites.

Sample ID / Date Sampled			Chromium	Lead	Zinc
Unit (dry weight)			mg/kg	mg/kg	mg/kg
LOR			0.1	0.1	0.2
ZERO CONTROLS	ZC1	19/04/2011	0.76	0.3	180
	ZC2	19/04/2011	0.76	0.21	150
	ZC3	19/04/2011	0.59	0.20	110
	ZC4	19/04/2011	0.69	0.23	140
	ZC5	19/04/2011	0.55	0.22	180
	Mean		0.67	0.23	152
	StDev		0.10	0.04	29.5
	Median		0.69	0.22	150
	Min		0.55	0.20	110
	Max		0.76	0.30	180
IMPACT SITES	Bow1	30/05/2011	1.9	0.33	150
	Bow2	30/05/2011	0.81	0.31	150
	Bow3	30/05/2011	1	0.28	210
	Mid1	30/05/2011	1.4	0.36	130
	Mid2	30/05/2011	1.1	0.43	260
	Mid3	30/05/2011	1.9	0.41	220
	Stern1	30/05/2011	2.1	0.41	170
	Stern2	30/05/2011	1.2	0.38	180
	Stern3	30/05/2011	1	0.33	130
	Mean		1.4	0.36	178
	StDev		0.47	0.05	44.4
	Median		1	0.36	170
	Min		0.81	0.28	130
	Max		2.1	0.43	260

4. DISCUSSION

Aquatic invertebrates, in particular, bivalve molluscs, are commonly used as test organisms in active biomonitoring studies (Bervoets *et al.* 2003). Bivalve molluscs are filter feeding organisms which actively filter dissolved and suspended matter from the water by pumping water through specialised filtration structures. Filter feeders play a significant role in the removal of toxins and bacteria from the water column and so are suitable organisms to test for water contamination and the accumulation of contaminants or toxins in the tissues of marine organisms (Huber 2010).

Previous studies have utilised transplanted mussels effectively to assess the bioavailability of metals and other contaminants. A variety of mussel species, including the Blue Mussel, *M. edulis*, and the Zebra Mussel, *Dreissena polymorpha*, have been used in Australia and overseas (respectively) to assess potential water quality contamination by micro-contaminants and heavy metals and its potential impact on marine organisms (e.g. Haynes and Toohey 1998; Giusti *et al.* 1999; Romeo *et al.* 2003; Bervoets *et al.* 2005a, 2005b; Smolders *et al.* 2005).

This technique is based on the generic study known as “Mussel Watch” which is widely used in Europe and the United States where mussels are abundant (Scanes 1991). The approach applied in this study is based on active bio-monitoring (ABM) where the mussel samples are sourced from one population at one location and translocated to test sites, ensuring comparable biological samples (de Kock and Kramer 1994).

Although an increase in metal concentrations has been observed between the transplanted mussels and the mussels placed near the vessel, the significant increase noted for lead and chromium cannot be directly attributed to the presence of the vessel without consideration of reference concentrations. Without data from the two reference areas, results remain confounded.

5. REFERENCES

Bervoets L., Smolders R., Voets J., and Blust R. (2003). *Active biomonitoring (ABM) using aquatic invertebrates.* In: Biological evaluation and monitoring of the quality of surface waters. Ed. Symoens, J.J. Brussels, KBIN. pg 71-76.

Bervoets L., Voets J., Covaci A., Chu S., Qadah D., Smolders R., Schepens P., and Blust R. (2005a) Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of microcontaminants in Flemish surface waters. *Environmental Science and Technology* **39**, 1492-1505.

Bervoets, Lieven; Voets, Judith; Smolders, Roel; Blust, Ronny (2005b). Metal accumulation and condition of transplanted zebra mussel (*Dreissena polymorpha*) in metal polluted rivers. *Aquatic Ecosystem Health and Management* **8**, 451-460.
Bulletin **36**, 396-399.

de Kock W. C. and Kramer K. J. M (1994). *Active biomonitoring (ABM) by translocation of bivalve molluscs*. In: Biomonitoring of coastal waters and estuaries. ed. K.J.M. Kramer. CRC Press, Boca Raton, pg. 51-84.

Giusti L., Williamson C., and Mistry A. (1999). Biologically available trace metals in *Mytilus edulis* from the coast of Northeast England. *Environmental International* **25**, 969-981.

Haynes D., and Toohey D. (1998). The use of transplanted, cultured mussels (*Mytilus edulis*) to
Huber M. (2010). *Compendium of Bivalves. A Full-color Guide to 3'300 of the World's Marine Bivalves. A Status on Bivalvia after 250 Years of Research*. Hackenheim: Conch Books. pp. 901 pp.
MacLeod I., Morrison P., Richards, V., and West, N. (2004). Corrosion monitoring and the environmental impact of decommissioned naval vessels as artificial reefs. *Proceedings of Metals 2004*, National Museum of Australia. pg 53-74.
monitor pollutants along the Ninety Mile Beach, Victoria, Australia III. Heavy metals. *Marine Pollution*

Roméo M., Hoarau P., Garello G., Gnassia-Barelli M., and Girard J. (2003). Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. *Environmental Pollution* **122**, 369-378.

Scanes P. (1991). *Inshore Bioaccumulation Studies along the Sydney Coast*. In: Proceedings of a Bioaccumulation Workshop, Assessment of the Distribution, Impacts and Bioaccumulation of Contaminants in Aquatic Environments. eds. Miskiewicz A.G. Sydney Water Board and Australian Marine Sciences Association Inc.

Smolders R., Voets J., Bervoets L., Blust R., and Wepener V. (2005). *Active biomonitoring (ABM) by translocation of bivalve molluscs.* In: The encyclopedia of water: water quality and resource development . ed. Lehr, J. H. Hoboken, N.Y. Wiley-Interscience. pg. 33-37.

Appendix 1

Laboratory Sample Receipts and Reports of Analysis

SAMPLE RECEIPT NOTIFICATION

Attention : Katie Newton

Client : Worley Parsons Pty Ltd
3 Warabrook Blvd
Warabrook NSW 2304
Telephone : 02 4985 0020
Facsimile : 02 4985 0099

Project : Ex-HMAS Adelaide Monitoring **Order Number :**

Laboratory Reference : A11/1836

Completed Chain of Custody accompanied samples.	**YES**
Samples were received in good condition and correctly preserved for all tests.	**YES**
Samples were received in sufficient time to allow laboratory to meet holding times.	**YES**
Samples were received chilled/chilling (if required).	**YES**

Date samples received : **20/04/2011**
Matrix : **Mussels**
No. of samples : **5**
Scheduled reporting date : **3 May 11**

Client Services Manager : **Daniel Um**

Telephone : 02 9888 9077
Email : daniel.um@advancedanalytical.com.au

Contact your Client Services Manager for all queries and issues regarding this sample batch.

Note: Turnaround time begins at time of receipt at laboratory, surcharges may apply for fast turnaround.

Water samples will be appropriately stored for 1 month from date of receipt of samples.
Soil / Sediment samples will be appropriately stored for 3 months from date of receipt of samples.

COMMENTS:

Advanced Analytical Australia Pty Ltd
ABN 20 105 644 979
11 Julius Avenue
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

REPORT OF ANALYSIS

Laboratory Reference: A11/1836 [R00]

Client: Worley Parsons Pty Ltd
3 Warabrook Blvd
Warabrook NSW 2304

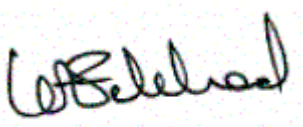
Contact: Katie Newton

Order No:
Project: Ex-HMAS Adelaide Monitoring
Sample Type: Mussels
No. of Samples: 5
Date Received: 20/04/2011
Date Completed: 28/04/2011

Laboratory Contact Details:

Client Services Manager: Daniel Um
Technical Enquiries: Ian Eckhard
Telephone: +61 2 9888 9077
Fax: +61 2 9888 9577
Email: daniel.um@advancedanalytical.com.au

Attached Results Approved By:



Ian Eckhard
Technical Director

Comments:
All samples tested as submitted by client. All attached results have been checked and approved for release.
This is the Final Report and supersedes any reports previously issued with this batch number.
This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. This document shall not be reproduced, except in full.

Accreditation No.15109

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Batch Number: A11/1836 [R00] **Project:** Ex-HMAS Adelaide Monitoring

Laboratory Reference: **Client Reference:** **Date Sampled:** **Analysis Description**	- - - **Method**	- - - **Units**	**A11/1836/1** **ZC1** **19/04/2011**	**A11/1836/2** **ZC2** **19/04/2011**	**A11/1836/3** **ZC3** **19/04/2011**	**A11/1836/4** **ZC4** **19/04/2011**
Trace Elements						
Chromium	04-008	mg/kg	0.76	0.76	0.59	0.69
Lead	04-008	mg/kg	0.30	0.21	0.2	0.23
Zinc	04-008	mg/kg	180	150	110	140

Laboratory Reference: **Client Reference:** **Date Sampled:** **Analysis Description**	- - - **Method**	- - - **Units**	**A11/1836/5** **ZC5** **19/04/2011**
Trace Elements			
Chromium	04-008	mg/kg	0.55
Lead	04-008	mg/kg	0.22
Zinc	04-008	mg/kg	180

Method	Method Description
04-008	Metals in food by ICP-OES, mg/kg

Result Comments

[<] Less than

[INS] Insufficient sample for this test

[NA] Test not required

Samples analysed on blended, freeze-dried mussels composites. Results are reported on this basis.

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Batch Number: A11/1836 [R00] **Project:** Ex-HMAS Adelaide Monitoring

QUALITY ASSURANCE REPORT

TEST	UNITS	Blank
Chromium	mg/kg	<0.1
Lead	mg/kg	<0.1
Zinc	mg/kg	<0.2

Comments:

RPD = Relative Percent Deviation
[NT] = Not Tested
[N/A] = Not Applicable
'#' = Spike recovery data could not be calculated due to high levels of contaminants

Acceptable replicate reproducibility limit or RPD:
Results < 10 times LOR: no limits.
Results >10 times LOR: 0% - 50%.

Acceptable matrix spike & LCS recovery limits:
Trace elements 70-130%
Organic analyses 50-150%
SVOC & speciated phenols 10-140%
Surrogates 10-140%

When levels outside these limits are obtained, an investigation into the cause of the deviation is performed before the batch is accepted or rejected, and results are released.

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia
Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

SAMPLE RECEIPT NOTIFICATION

Attention : Katie Newton

Client : Worley Parsons Pty Ltd
3 Warabrook Blvd
Warabrook NSW 2304
Telephone : 02 4985 0020
Facsimile : 02 4985 0099

Project : Ex-HMAS Adelaide Monitoring **Order Number :**

Laboratory Reference : A11/2497

Completed Chain of Custody accompanied samples.	**YES**
Samples were received in good condition and correctly preserved for all tests.	**YES**
Samples were received in sufficient time to allow laboratory to meet holding times.	**YES**
Samples were received chilled/chilling (if required).	**YES**

Date samples received : **31/05/2011**
Matrix : **Mussels**
No. of samples : **9**
Scheduled reporting date : **9 Jun 11**

Client Services Manager : **Daniel Um**

Telephone : 02 9888 9077
Email : daniel.um@advancedanalytical.com.au

Contact your Client Services Manager for all queries and issues regarding this sample batch.

Note: Turnaround time begins at time of receipt at laboratory, surcharges may apply for fast turnaround.

Water samples will be appropriately stored for 1 month from date of receipt of samples.
Soil / Sediment samples will be appropriately stored for 3 months from date of receipt of samples.

COMMENTS:

Advanced Analytical Australia Pty Ltd
ABN 20 105 644 979
11 Julius Avenue
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

REPORT OF ANALYSIS

Laboratory Reference: A11/2497 [R00]

Client: Worley Parsons Pty Ltd
3 Warabrook Blvd
Warabrook NSW 2304

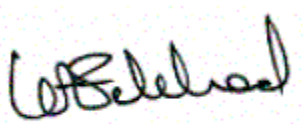
Contact: Katie Newton

Order No:
Project: Ex-HMAS Adelaide Monitoring
Sample Type: Mussels
No. of Samples: 9
Date Received: 31/05/2011
Date Completed: 7/06/2011

Laboratory Contact Details:

Client Services Manager: Daniel Um
Technical Enquiries: Ian Eckhard
Telephone: +61 2 9888 9077
Fax: +61 2 9888 9577
Email: daniel.um@advancedanalytical.com.au

Attached Results Approved By:



Ian Eckhard
Technical Director

Comments:
All samples tested as submitted by client. All attached results have been checked and approved for release.
This is the Final Report and supersedes any reports previously issued with this batch number.
This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. This document shall not be reproduced, except in full.

Accreditation No.15109

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Batch Number: A11/2497 [R00] **Project:** Ex-HMAS Adelaide Monitoring

Laboratory Reference:	-	-	**A11/2497/1**	**A11/2497/2**	**A11/2497/3**	**A11/2497/4**
Client Reference:	-	-	**Bow1**	**Bow2**	**Bow3**	**Mid1**
Date Sampled:	-	-	**30/05/2011**	**30/05/2011**	**30/05/2011**	**30/05/2011**
Analysis Description	**Method**	**Units**				
Trace Elements						
Chromium	04-008	mg/kg	1.9	0.81	1.0	1.4
Lead	04-008	mg/kg	0.33	0.31	0.28	0.36
Zinc	04-008	mg/kg	150	150	210	130

Laboratory Reference:	-	-	**A11/2497/5**	**A11/2497/6**	**A11/2497/7**	**A11/2497/8**
Client Reference:	-	-	**Mid2**	**Mid3**	**Stern1**	**Stern2**
Date Sampled:	-	-	**30/05/2011**	**30/05/2011**	**30/05/2011**	**30/05/2011**
Analysis Description	**Method**	**Units**				
Trace Elements						
Chromium	04-008	mg/kg	1.1	1.9	2.1	1.2
Lead	04-008	mg/kg	0.43	0.41	0.41	0.38
Zinc	04-008	mg/kg	260	220	170	180

Laboratory Reference:	-	-	**A11/2497/9**
Client Reference:	-	-	**Stern3**
Date Sampled:	-	-	**30/05/2011**
Analysis Description	**Method**	**Units**	
Trace Elements			
Chromium	04-008	mg/kg	1.0
Lead	04-008	mg/kg	0.33
Zinc	04-008	mg/kg	130

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia
Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Batch Number: A11/2497 [R00] **Project:** Ex-HMAS Adelaide Monitoring

Method	Method Description
04-008	Metals in food by ICP-OES, mg/kg

Result Comments

[<] Less than

[INS] Insufficient sample for this test

[NA] Test not required

Samples analysed on blended, freeze-dried mussels composites.

Results are reported on this basis.

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia

Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Batch Number: A11/2497 [R00] **Project:** Ex-HMAS Adelaide Monitoring

QUALITY ASSURANCE REPORT

TEST	UNITS	Blank	Duplicate Sm#	Duplicate Results	Spike Sm#	Spike Results
Chromium	mg/kg	<0.1	A11/2497-1	1.9 || 1.9 || RPD: 0	A11/2497-2	102%
Lead	mg/kg	<0.1	A11/2497-1	0.33 || 0.34 || RPD: 3	A11/2497-2	90%
Zinc	mg/kg	<0.2	A11/2497-1	150 || 150 || RPD: 0	A11/2497-2	103%

Comments:

RPD = Relative Percent Deviation
[NT] = Not Tested
[N/A] = Not Applicable
'#' = Spike recovery data could not be calculated due to high levels of contaminants

Acceptable replicate reproducibility limit or RPD: Results < 10 times LOR: no limits.
Results >10 times LOR: 0% - 50%.

Acceptable matrix spike & LCS recovery limits: Trace elements 70-130%
Organic analyses 50-150%
SVOC & speciated phenols 10-140%
Surrogates 10-140%

When levels outside these limits are obtained, an investigation into the cause of the deviation is performed before the batch is accepted or rejected, and results are released.

Advanced Analytical Australia Pty ltd
ABN 20 105 644 979
11 Julius Avenue,
North Ryde NSW 2113 Australia
Ph: + 61 2 9888 9077
Fax: + 61 2 9888 9577
contact@advancedanalytical.com.au
www.advancedanalytical.com.au

Appendix 2

Output of Statistical Analysis

Statistical Analysis Output – Statistica Version 5

1-Way ANOVA

Difference in metal concentrations between time 0 (zero controls) and time 1 (6 weeks)

Summary of all Effects; design: (mussel contam data.sta)
1-TIME

	Wilks' Lambda	Rao's R	df 1	df 2	p-level
1	.315712	7.224812	3	10	.007286

Tukey HSD test; variable CHROMIUM (mussel contam data.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1}	{2}
		.6700000	1.378889
0	{1}		.006954
1	{2}	.006954	

Tukey HSD test; variable LEAD (mussel contam data.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1}	{2}
		.2320000	.3600000
0	{1}		.000571
1	{2}	.000571	

Tukey HSD test; variable ZINC (mussel contam data.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1}	{2}
		152.0000	177.7778
0	{1}		.270971
1	{2}	.270971	

1-Way ANOVA

Difference in metal concentrations between the impact sites at 6 weeks

Summary of all Effects; design: (mussel contam data2.sta)
1-TIME

	Wilks' Lambda	Rao's R	df 1	df 2	p-level
1	.196836	1.671955	6	8	.244884

Tukey HSD test; variable CHROMIUM (mussel contam data2.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1} 1.236667	{2} 1.466667	{3} 1.433333
1	{1}		.859761	.894745
2	{2}	.859761		.996807
3	{3}	.894745	.996807	

Tukey HSD test; variable LEAD (mussel contam data2.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1} .3066667	{2} .4000000	{3} .3733333
1	{1}		.037147	.120929
2	{2}	.037147		.633291
3	{3}	.120929	.633291	

Tukey HSD test; variable ZINC (mussel contam data2.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

		{1} 170.0000	{2} 203.3333	{3} 160.0000
1	{1}		.666591	.961929
2	{2}	.666591		.518976
3	{3}	.961929	.518976	